Plant biomass, not plant economics traits, determines responses of soil CO 2 efflux to precipitation in the C 4 grass Panicum virgatum

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Abstract: Plant responses to major environmental drivers like precipitation can influence important aspects of carbon (C) cycling like soil CO2 efflux (urn:x-wiley:00220477:media:jec13382:jec13382-math-0001). These responses may be predicted by two independent classes of drivers: plant size—larger plants respire more and produce a larger quantity of labile C, and plant economics—plants possessing more acquisitive plant economics strategies (i.e. high metabolic rate and tissue nutrient content) produce higher-quality tissue that respires rapidly and decomposes quickly. At two sites in central Texas, USA with similar climates and differing soil characteristics, we examined the response of eight Panicum virgatum genotypes to three annual precipitation levels defined by the driest, average and wettest years from each site's precipitation history. We evaluated the individual and joint influence of plant genotypes and precipitation on urn:x-wiley:00220477:media:jec13382:jec13382-math-0002 and traits related to plant economics and plant size. We then used confirmatory path analysis to evaluate whether effects of precipitation on urn:x-wiley:00220477:media:jec13382:jec13382-math-0003 were in part related to effects of precipitation on plant economics traits or size ('mediated' effects). These genotypes exhibited variation in plant economics traits and above-ground net primary productivity (ANPP), an above-ground measure of plant size. Increasing precipitation increased urn:x-wiley:00220477:media:jec13382:jec13382-math-0004 and ANPP more than plant economics traits. At both sites, ANPP was the best predictor of urn:x-wiley:00220477:media:jec13382:jec13382-math-0005 . Moreover, the sites differed in the ways that plant size and plant economics traits combined with precipitation to influence urn:x-wiley:00220477:media:jec13382:jec13382-math-0006 . At the Austin site, the positive effect of precipitation on urn:x-wiley:00220477:media:jec13382:jec13382-math-0007 was mediated primarily by ANPP, offset by a smaller effect of leaf nitrogen content; no direct precipitation effect was detected. At the Temple site, increasing precipitation had positive direct and ANPP-mediated effects on urn:x-wiley:00220477:media:jec13382:jec13382-math-0008 . This suggests that greater water limitation at Austin may strengthen the links between plant size and urn:x-wiley:00220477:media:jec13382:jec13382-math-0009 . Synthesis. Estimates of C cycling can be improved by accounting for mediation of precipitation effects on urn:x-wiley:00220477:media:jec13382:jec13382-math-0010 by plant economics traits and plant size in resource-limited environments.

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Plant biomass, not plant economics traits, determines responses of soil CO_2 efflux to precipitation in the C_4 grass *Panicum virgatum*

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Abstract

1. Plant responses to major environmental drivers like precipitation can influence important aspects of carbon (C) cycling like soil CO_2 efflux (J_{CO2}). These responses may be predicted by two independent classes of drivers: plant size—larger plants respire more and produce a larger quantity of labile C, and plant economics—plants possessing more acquisitive plant economics strategies (i.e., high metabolic rate and tissue nutrient content) produce higher-quality tissue that respires rapidly and decomposes quickly.
2. At two sites in central Texas, USA with similar climates and differing soil characteristics, we examined the response of eight *Panicum virgatum* genotypes to three annual precipitation levels defined by the driest, average, and wettest years from each site's precipitation history. We evaluated the individual and joint influence of plant genotypes and precipitation on J_{CO2} and traits related to plant economics and plant size. We then used confirmatory path analysis to evaluate whether effects of precipitation on J_{CO2} were in part related to effects of precipitation on plant economics traits or size ('mediated' effects).
3. These genotypes exhibited variation in plant economics traits and aboveground net primary productivity (ANPP), an aboveground measure of plant size. Increasing precipitation increased J_{CO2} and ANPP much more than plant economics traits. At both sites, ANPP was the single best predictor of J_{CO2}. Moreover, the sites differed in the ways that plant size and plant economics traits combined with precipitation to influence J_{CO2}. At the Austin site, the positive effect of precipitation on J_{CO2} was mediated primarily by ANPP, offset by a smaller effect of leaf nitrogen content; no direct precipitation effect was detected. At the Temple site, increasing precipitation had positive direct and ANPP-mediated effects on J_{CO2}. This

suggests that greater water limitation at Austin may strengthen the links between plant size and J_{CO2}.

4. *Synthesis* Estimates of carbon cycling can be improved by accounting for mediation of precipitation effects on J_{CO2} by plant economics traits and plant size in resource-limited environments.

Introduction

Soil CO_2 efflux (J_{CO2}) is a major component of the terrestrial carbon (C) cycle and is the main flux of C from the biosphere to the atmosphere (Schlesinger & Andrews 2000). J_{CO2} combines CO_2 respired from autotrophic and heterotrophic sources (Hanson *et al.* 2000; Schlesinger & Andrews 2000). Both sources depend on carbon assimilation, and thus are mechanistically linked to the traits of the Plant Economics Spectrum (PES). The PES describes a continuum of covarying resource acquisition and allocation traits ranging from acquisitive plants with high metabolic rates and tissue nitrogen (N) content to conservative plants with lower metabolic rates and tissue N content (Freschet *et al.* 2010; Reich 2014). Plants with acquisitive PES strategies support roots that respire more and release proportionately more labile C (Tjoelker *et al.* 2005; Roumet *et al.* 2016). Moreover, acquisitive strategies are also linked to higher leaf and root N content (Freschet *et al.* 2010; Roumet *et al.* 2016), resulting in high-quality litter that decomposes more rapidly (Cornwell *et al.* 2008). J_{CO2} is also mechanistically linked to plant size—larger plants produce more belowground biomass (Shipley & Meziane 2002), increasing the mass of respiring roots and litter inputs to soil C cycling. A trait-based approach to understanding the biotic controls on J_{CO2} can yield insights into the links between traits and ecosystem processes (De Long *et al.* 2019; Fry *et al.* 2019). Specifically, this approach

may identify covarying aboveground and belowground traits that predict belowground processes, but these links remain poorly understood.

Soil CO_2 efflux is strongly affected by abiotic factors, including temperature, precipitation, and edaphic properties. Root and microbial respiration are highly sensitive to temperature, which is often reflected in the seasonal trajectories of J_{CO2} (Wang *et al.* 2014; Dacal *et al.* 2019). J_{CO2} also typically increases with increasing precipitation; increased soil moisture enhances soil microbial activity and reduces plant water stress, increasing C assimilation and primary productivity (Hoover, Knapp & Smith 2016; Deng *et al.* 2017). The effects of precipitation and temperature on J_{CO2} are mediated by soil edaphic properties, especially soil texture. Finer-textured soils have higher water holding capacity and higher soil organic matter content than coarse-textured soils (Weil & Brady 2016). As a result, at a given amount of precipitation, plant size and J_{CO2} may often be higher on finer compared to coarser soils (Bouma & Bryla 2000; Cable *et al.* 2008).

Many species show a high degree of intraspecific trait variation (Siefert *et al.* 2015). Across broad environmental gradients, intraspecific trait variation can reflect local adaptation to resource availability and other environmental factors (Kawecki & Ebert 2004; Anderson, Willis & Mitchell-Olds 2011). Because of this local adaptation, within genotypes PES traits may respond little to changes in abiotic conditions, such as precipitation, compared to the range of trait variation among genotypes (Mason & Donovan 2015). Therefore, genotypes from varying locally adapted populations can provide variation in trait expression and plant size from which to test how traits and size influence J_{CO2}.

The common C_4 grass *Panicum virgatum* L. varies considerably in plant size and PES traits across its native range in the central North American grasslands (Casler 2012). Northern

69 genotypes often exhibit acquisitive strategies; southern genotypes typically exhibit conservative
70 strategies (Aspinwall *et al.* 2013). Moreover, variation in several important functional traits is
71 highly heritable and correlated with climate of genotype origin (Aspinwall *et al.* 2013),
72 potentially limiting how much these traits respond to precipitation. If precipitation influences
73 plant size and PES traits differently, the relative influence of each on J_{CO2} should determine how
74 J_{CO2} changes with precipitation (Whitham *et al.* 2006; Bailey *et al.* 2009).
75 We examined the effects of precipitation and plant size and PES trait variation on J_{CO2} in
76 eight *Panicum virgatum* genotypes collected from a north-south climatic gradient. These
77 genotypes were established in two common gardens in central Texas, one at a site with deep,
78 fine-textured soils, the other on a site with shallow, coarse-textured soils. These genotypes
79 display a range of PES traits correlated with the temperature in their habitat of origin (Aspinwall
80 *et al.* 2013), and also vary in how strongly their productivity increases with precipitation
81 (Aspinwall *et al.* 2017). Here we examine three predictions concerning the interrelation of plant
82 functional traits, plant productivity, and ecosystem carbon cycling.
83 1. J_{CO2} will increase with increasing precipitation across *P. virgatum* genotypes that vary in
84 size and in position along the acquisitive to conservative continuum of PES traits.
85 2. J_{CO2} will increase with plant size and with more acquisitive PES traits.
86 3. Plant size will be more responsive to precipitation than PES traits, therefore we expect
87 plant size to be the stronger mediator of precipitation effects on J_{CO2}.
88 **Methods**
89 This study was performed at two sites ~ 110 km apart in central Texas, USA: the USDA
90 Grassland, Soil and Water Research Lab near Temple, TX, USA on a deep (50-100 cm) fine-
91 textured soil (Austin silty clay, fine silty, carbonatic, Udorthentic Haplustol), and the Lady Bird

Johnson Wildflower Center near Austin, TX, USA on a shallow (35-50 cm) coarse-textured soil (Speck clay loam, clayey, thermic Lithic Argiustoll). The sites have similar climates. For Temple, mean annual precipitation is 910 mm, mean maximum temperature (July-August) is 35.0°C, and mean minimum temperature is 3.0°C. At Austin, mean annual precipitation is 870 mm, mean maximum temperature (July-August) is 35.0°C, and mean minimum temperature is 5.6°C.

At each site, treatments were assigned in a randomized complete block design across twelve 5×5 m plots arranged in four spatial blocks. Plots within blocks were 0.25 m apart and blocks were 2.76 m apart. Pond liner (1.84 mm thick; Firestone Specialty Products, Indianapolis, IN, USA) surrounded each plot to limit the movement of subsurface water and roots between plots. The pond liner extended 10 cm above the soil surface to limit overland flow of water into and out of plots, and extended to a depth of 120 cm at the Temple site and 20 cm at the Austin site, reflecting differences in soil depth. The plots were arranged beneath 18.3×73.0 m rainout shelters (Windjammer Cold Frame, International Greenhouse Company, Danville, IL, USA) covered with 150 micron polyethylene greenhouse film. The sides of these shelters were open (2.1 m high walls with 4.2 m high eaves on both ends) to maximize air movement and heat dissipation. The shelters excluded natural rainfall year-round (Aspinwall *et al.* 2013). Precipitation treatments were applied using 90° sprinklers (Hunter HP2000, Hunter Industries Inc., San Marcos, CA, USA) attached to 1 m risers in all four corners of each experimental plot. The sprinklers were operated by a programmable controller (LEIT XRC Series Ambient Powered Irrigation Controller, DIG Corporation, Vista, CA, USA).

Precipitation treatments

Precipitation treatments began in March 2012 and continued throughout 2013. Plots were assigned to one of three precipitation treatments representing the effects of severe drought, a year of average precipitation, or an extremely wet year at each site. Precipitation treatments were defined from the historical precipitation record of each site. Specifically, the low precipitation treatment was the average of the ten driest years on record at each site, the mean treatment was the average of the ten years nearest the mean, and the high precipitation treatment was the average of the ten wettest years on record at each site (coarse-textured site: 1938-2010; fine-textured site: 1900-2002). At Temple, this corresponded to annual precipitation amounts from 530 to 1541 mm; at Austin, from 349 to 1330 mm (Table S1). The sequence of experimental rainfall events for each treatment was produced using a stochastic weather generator, LARS-WG 5.5 (Semenov *et al.* 1998), which was calibrated using the precipitation records at each site (Aspinwall *et al.* 2017). The generated rainfall sequences approximated the selected sets of years in seasonality, size distribution, and spacing of rainfall events. To quantify the severity of these treatments, we calculated potential evapotranspiration and SPEI using the SPEI package in R (Beguería & Vicente-Serrano 2017). Demand for water exceeded supply in the low and mean treatments at both sites (Table S1, Fig S1). Additionally, SPEI-6 measured in October indicates that the high and low precipitation treatments simulated conditions extreme enough to be expected to occur only 1-2 times in 20 years (Table S1).

Genotypes

Common gardens at each site were planted with eight *Panicum virgatum* L. genotypes originating between 27° N and 35° N, spanning the U.S. Central Plains states of Texas and Oklahoma (Table S2). Individuals of each genotype were clonally propagated from an individual genet collected at each location. Clones of each genotype were planted in 2011 on 1 m centers in

duplicate in each plot, constrained so that duplicates were never adjacent. Plants were well-watered during establishment. We randomly selected one individual of each genotype for study. Thus, our study initially comprised 192 individual plants (2 sites × 3 precipitation treatments × 4 spatial blocks × 8 genotypes). Due to mortality and missing data on some responses, our final data set included 162 individuals.

Data collection

We measured J_{CO2} through the 2013 growing season. Specifically, we measured J_{CO2} once during each month between May and November, except July and October at Temple and once during each month between June and November, except August and October at Austin. Measurements were taken at points defined by PVC collars (1.7 cm height, 8 cm diameter) which were installed one month prior to the start of measurements. Collars were placed as close as practical to the north-facing base of each plant in order to minimize influences from roots originating in adjacent plants. J_{CO2} was measured with infrared gas analyzers (Temple: Li-COR 6400 fitted with a 6400-09 soil respiration chamber, Austin: Li-COR 8100 automated soil CO_2 flux system fitted with 8100-102 survey chamber; Li-COR Biosciences, Inc, Lincoln, NE, USA). The instruments share the same theoretical approach to measurement but have slight differences in implementation that are unlikely to create substantive differences in measurement of precipitation or genotype responses between the two sites (McDermitt *et al.* 2005). Specifically, in Austin, all measurements began with 20 s dead time, followed by a 2 min observation with 2 consecutive measurements per plant and no delay between measurements, then 30 s purge time. Chamber offset was set to the actual height of the collar based on the average of 3 measurements taken around the edge. In Temple, chamber [CO_2] was reduced 5-10 ppm below ambient (~380 ppm). Once the rate of [CO_2] increase stabilized (usually within ~ 30 s), J_{CO2} was logged until

160 the chamber [CO_2] had increased to 5-10 ppm above ambient, usually 20-30 s. Soil water content
161 and soil temperature (0–10 cm) were measured concurrently using hand-held probes (Temple:
162 Fieldscout TDR 200, Spectrum Technologies, Plainfield, Illinois, USA; HH84 temperature
163 probe, Omega Engineering Inc., Norwalk, CT, USA; Austin: ML2 Theta Probe, Dynamax Inc.,
164 Houston, TX, USA; E type temperature probe, Omega Engineering Inc. Norwalk, CT, USA). To
165 best highlight the links between precipitation, plant traits, and ecosystem function, J_{CO2}
166 measurements were always conducted between 0900 and 1400. Plants were sampled in random
167 order. Each monthly sampling required two to four days at each site to complete. These monthly
168 J_{CO2} measurements were reduced to a single value per plant by calculating the area under the
169 curve of monthly measurements, rather than an arithmetic mean. This avoids bias caused by the
170 differing pattern of missing months between the sites. We retained the maximum soil
171 temperature measured for each plant, which was recorded during July at Austin and August at
172 Temple.

173 We measured two foliar traits—foliar nitrogen content (N_{MASS}) and leaf dry matter
174 content (LDMC)—in early August 2013, during the portion of the season when plants experience
175 maximum water stress. To quantify N_{MASS}, two or three young, fully emerged leaves per plant
176 were dried and ground to a fine powder, then combusted in an elemental analyzer (Flash 2000
177 Organic Elemental NC Analyzer, Waltham, MA, USA). Leaf dry matter content was estimated
178 from the ratio of the oven-dry mass to water-saturated mass of young, fully emerged leaves
179 (Pérez-Harguindeguy *et al.* 2013). Leaves were rehydrated by placing the cut end of the leaf in a
180 30-ml plastic tube with 4-5 ml of deionized water for four hours in a cool, dark room. Leaves
181 were then weighed, dried at 65ºC for at least 48 hours, and re-weighed. N_{MASS} measured in this
182 study spanned the 3^{rd}-37^{th} percentiles of global N_{MASS} reported by Wright *et al.* (2004).

We measured plant size as current year aboveground biomass production for each plant (kg plant^{-1}), an estimate of ANPP. We harvested biomass by clipping at 10 cm above the soil surface in early December 2013, then weighing the biomass after drying at 65ºC for at least 48 h in forage drying ovens.

To assess belowground traits, we buried root ingrowth cores (Li *et al.* 2012; Ontl *et al.* 2013) adjacent to one randomly selected individual of each genotype in each plot. Each 5 cm diameter × 15 cm deep core was constructed of 2 mm LLDPE plastic mesh (Darice®, Strongsville, OH, USA) with a solid bottom and filled with sieved, root-free field soil at local bulk density. The mass of roots filling the ingrowth core during the burial period provide an estimate of belowground primary production (BNPP). At both sites, root cores were buried in March 2013; cores were removed in July 2013 at Austin and in October 2013 at Temple. Upon removal, we separated roots from soil using tweezers, washed the roots of any remaining soil, and weighed the roots after drying at 65ºC. BNPP was expressed per volume of soil.

Root C and N were measured to quantify the root C:N ratio. We ground the dried roots that were removed from the ingrowth cores to a fine powder and measured C and N content using the same protocol as with foliar N_{MASS}. Because of differing burial durations, BNPP is not directly comparable between sites, but is comparable among precipitation treatments within sites.

Data analysis

We evaluated the relationships among precipitation, J_{CO2}, soil moisture, PES traits, and plant size using two approaches. First, we fit linear mixed-effect models (LMMs) that included plant genotype as a categorical predictor, annual precipitation applied as a continuous predictor, and interactive effects of these two predictors. Individual plants were nested within plots and plots were nested within blocks. Models were fit using the `lme` function in the nlme package

(Pinheiro *et al.* 2016) in R version 3.3.2. Variables were natural log transformed to meet distributional assumptions. We ran separate models at each site. We also performed repeated-measures analyses on soil moisture and J_{CO2} similar to the models described above with three differences: all values in the soil moisture and J_{CO2} time series were included as responses, month was an additional predictor, and an AR1 correlation structure was added. To identify the extent to which plant size and PES traits covaried, we performed exploratory factor analysis using the `factanal` function with the varimax rotation.

Second, to reveal in detail how specific PES traits and plant size may have mediated the effects of precipitation on J_{CO2}, we performed piecewise structural equation modelling (piecewise SEM) (Shipley 2009) using the piecewiseSEM package (Lefcheck 2016). Piecewise SEM uses LMMs to estimate each path, allowing us to incorporate random effects and correlation structures and also to accommodate smaller datasets than possible in standard SEM (Shipley 2009; Lefcheck 2016). A strength of structural equation models is the ability to distinguish direct effects of a predictor variable on a response of interest from 'mediated' effects, where the predictor variable affects the response of interest by affecting the response of a third variable which is also related to the response of interest.

We devised SEMs containing causal paths linking precipitation with J_{CO2} and each PES trait and size variable to evaluate direct effects of precipitation, and linking plant variables to J_{CO2}, to resolve the indirect effects of precipitation on J_{CO2} mediated by the plant variables. We further included paths linking soil temperature with ANPP and J_{CO2} because of typically strong temperature effects on J_{CO2}. Separate SEMs were fit for each site.

LMMs for each path in the SEM were fit using the `lme` function in the nlme package (Pinheiro *et al.* 2016). Each LMM included a random effect of plant nested within plot nested

within block. We simplified each SEM to remove non-significant paths. Using the `sem.coefs` function in piecewiseSEM, we standardized each variable in the causal model to mean = 0 and standard deviation = 1. Standardized path coefficients were estimated by the LMM regression coefficients. Indirect effects were estimated by multiplying the standardized path coefficients. All endogenous variables were natural log transformed prior to standardization.

We assessed the goodness of fit of the SEMs with a test of directed separation (d-sep test, sensu Shipley 2009) using the `sem.fit` function in the piecewiseSEM package. The d-sep test evaluates whether any necessary paths are missing from the model (Lefcheck 2016). This is necessary because the goodness of fit tests used in standard SEM are inappropriate in piecewiseSEM (Shipley 2009).

Finally, to evaluate the relative strength of aboveground and belowground plant variables in predicting J_{CO2}, we performed relative variable importance analysis. We applied the LMM model described above to all possible additive combinations of the plant size and PES traits as predictors of J_{CO2} using the `dredge` function in MuMIn (Barton 2018). The `importance` function in MuMIn then estimates the relative importance of a variable from the sum of Akaike weights of all models in which a variable was included (Burnham & Anderson 2003).

Results

Consistent with our first prediction, precipitation was the primary driver of J_{CO2} and ANPP. J_{CO2} increased 130% between the lowest and highest precipitation levels at Austin (P=0.01; Table S3a, Fig 1a) and by 59% at Temple (P=0.001; Table S3b, Fig 1b). Similarly, ANPP increased with precipitation at both sites (Austin: 711% increase, P=0.004, Table S3a, Fig 1c; Temple: 191% increase, P<0.001, Table S3b, Fig 1d). BNPP increased at Austin (139% increase, P=0.02, Table S3a, Fig 1e), although not at Temple (26% increase, P=0.42, Table S3b,

Fig 1f). Larger responses to precipitation in Austin than in Temple are consistent with greater water limitation of plant size and J_{CO2}.The effect of precipitation on J_{CO2} also changed over time. At Austin, J_{CO2} was greater in mean and high than in the low treatment until the end of the growing season (November) when treatment differences in J_{CO2} declined (Precipitation × Time: $P<0.001$; Table S4a; Fig. S2a). The seasonal dynamic of J_{CO2} was similar at Temple, where high precipitation generally increased J_{CO2} compared to the mean and low treatments during August-September, with smaller differences early and late in the season ($P<0.001$; Table S4b; Fig. S2b).

Genotypic differences in J_{CO2} and plant size were smaller and less consistent. J_{CO2} did not differ between genotypes at either site (Austin: $P=0.08$; Temple: $P=0.21$, Table S3a), nor did BNPP (Austin: $P=0.46$, Table S1a; Temple: $P=0.38$, Table S3a). Aboveground biomass, however, varied strongly among genotypes, and to different degrees between sites. Genotypes varied by 57% in Austin ($P<0.001$, Table S3a) and by 104% in Temple ($P<0.001$, Table S3a). No significant genotype-by-precipitation effects were found.

Precipitation also influenced other abiotic variables. In Austin, soil moisture averaged ~10% in the low treatment but did not increase above ~15% in mean and high treatments (44% increase in Austin ($P=0.01$, Table S2a, Fig S3a). In Temple, soil moisture increased from ~20% in the low treatment to 30% in the high treatment at Temple (48% increase, $P<0.001$, Table S3b, Fig S3b). Soil temperature decreased with increased precipitation at both sites (Austin: $P<0.001$, Table S3a, Fig S3c; Temple: $P=0.004$, Table S3b, Fig S3d). Genotypes varied significantly in both soil moisture and temperature at Austin (genotypes varied by 22% in soil moisture, $P=0.04$; genotypes varied by 3% in temperature, $P=0.02$; Table S3a), but not at Temple (soil moisture: $P=0.13$; soil temperature: $P=0.13$; Table S3b).

The effect of precipitation treatment on soil moisture also changed over time (Austin: $P<0.001$; Temple: $P<0.001$; Table S4a,b). At Temple, in May and June soil moisture was 19-32% higher in the mean and high treatments than in the low treatment. Later (August-September), soil moisture was 72-139% higher in the high treatment than the mean and low treatments (Fig. S2d). At Austin, treatment differences were more idiosyncratic—in June, the mean treatment had 46-48% higher moisture than the low and high treatments. By September, the mean and high treatments had 66-103% higher soil moisture than the low treatment (Fig. S2d). The high treatment had considerably higher soil moisture than the other two treatments through the hottest portion of the study (July, August, September) in Temple, but not in Austin.

The genotypes provided sizeable variation in PES traits at both sites, while the effect of precipitation varied among traits (Table S3). For example, at both sites genotypes varied by 40% in LDMC (Austin: $P<0.001$, Table S3a, Fig 2a; Temple: $P<0.001$, Fig 2b), while precipitation did not influence LDMC (Austin: $P=0.61$; Temple: $P=0.96$). Precipitation interacted with genotype to influence N_{MASS} (Austin: $P = 0.02$, Table S3a, Fig 2c; Temple: $P = 0.004$, Table S3a, Fig 2d): N_{MASS} of most genotypes declined with precipitation (1-40% decline in Austin; 9-27% decline in Temple), while N_{MASS} increased with precipitation in two genotypes (increasing by 2-13% in Austin and by 5-32% in Temple). Two genotypes at Austin changed by less than 1%. Conversely, root C:N was influenced by both precipitation and genotype. Genotypes varied in root C:N by 64% at Austin ($P=0.01$, Table S3a, Fig 2e) and by 81% at Temple ($P=0.02$, Fig 2f); precipitation increased root C:N by 78% at Austin ($P<0.001$) and by 77% at Temple ($P=0.01$). Together, these results are consistent with our third prediction, that plant size will be more responsive to precipitation than PES traits.

PES traits covaried largely independently from plant size. In exploratory factor analysis, high Factor 1 scores were associated with large plant size, primarily ANPP and BNPP. High Factor 2 scores were associated with acquisitive plant economics traits, primarily, high foliar N_{MASS} and low LDMC (Fig 3a, Fig 3b). A fifth trait, root C:N, was the exception to this dichotomy, loading on both factor axes at both sites. The total variance explained by the two factors was similar for each site (Austin: χ^2=0.49, P=0.48; Temple: χ^2=1.44, P=0.23), but the plant size factor (Factor 1) explained twice as much variation as the traits factor (Factor 2) at Austin (0.349 and 0.171, respectively), but only 20% more variation at Temple (0.261 and 0.212, respectively).

SEMs

Both of the simplified SEMs adequately fit the data (Austin: Fisher's C=40.88, DF=28, P=0.055; Temple: Fisher's C=19.96, DF=18, P=0.335). At Austin, the total effect of precipitation on J_{CO2} was 0.57 (Table 1; Fig. 4a), composed of effects mediated by soil temperature (effect=0.36; Table S5a; Fig. S4a) and ANPP (effect=0.28; Table S5a; Fig. S4b,c), offset by a much weaker plant economics effect that only included a significant effect of N_{MASS} (effect=-0.06; Table S5a; Fig. S4d,e). No direct precipitation – J_{CO2} effect was resolved. In addition, J_{CO2} increased with LDMC independent of precipitation (effect=0.19; Table S4a; Fig. S4f).

At Temple, precipitation caused large direct (effect=0.53; Table S5b; Fig. S5a) and small ANPP-mediated (effect=0.09; Table S5b; Fig. S5b,c) effects on J_{CO2} (Table 1; Fig. 4b). Unlike Austin, neither plant economics nor abiotic factors mediated the effect of precipitation on J_{CO2} at Temple. N_{MASS} and LDMC independently predicted increased J_{CO2} (combined effect=0.52; Table S5b; Fig. S5d,e).

The SEMs did not resolve belowground-mediated effects of precipitation on J_{CO2}. Precipitation effects on BNPP and belowground traits occurred at both sites; in Austin, both root C:N ($P<0.001$; Table S5a; Fig. S4g) and BNPP ($P=0.02$; Table S5a; Fig. S4h) increased with precipitation; at Temple, root C:N increased with precipitation ($P=0.005$; Table S5b; Fig. S5f). However, paths relating these variables to J_{CO2} were not significant. These SEM results are partly consistent with our second prediction: J_{CO2} increased with increasing plant size and in some instances with more acquisitive PES traits. Additionally, these results are fully consistent with our third prediction: plant size was more responsive to precipitation than PES traits and consequently, was the stronger mediator of precipitation effects on J_{CO2}.

Variable Importance Analysis

Variable importance analysis reinforced the findings of the SEMs. At both sites, the aboveground predictors of J_{CO2} were superior to the belowground predictors (Austin: $\Delta AICc=17.5$; Temple: $\Delta AICc=9.3$), indicating that the weight of evidence for the aboveground predictors is > 6000× higher than for the belowground predictors at Austin and > 100× higher at Temple. Variable importance in predicting J_{CO2} ranked in the same order at both sites: ANPP, LDMC, foliar N_{MASS}, root C:N, BNPP (Table 2). Taken with the SEMs, these results indicate that aboveground traits were better predictors of J_{CO2} in this study.

Discussion

In this study, we examined how J_{CO2} responded to precipitation in *P. virgatum* genotypes that varied in size and in covarying resource acquisition and allocation traits related to the PES at two sites that differed in water limitation of plant productivity. We used structural equation modelling to understand the most influential relationships among covarying traits potentially mediating the effect of precipitation on J_{CO2}. This approach established that: 1) Precipitation

342 strongly influenced J_{CO2} at both sites. At Austin, precipitation more strongly limited ANPP,
343 which in turn mediated the effect of precipitation on J_{CO2}; at Temple, J_{CO2} was related to
344 precipitation through both direct and ANPP-mediated effects. 2) PES traits, including above- and
345 belowground traits related to growth and carbon gain, played a minor role in mediating
346 precipitation effects on J_{CO2}, although these traits influenced J_{CO2} independently of precipitation.
347 Our results aligned with other studies finding increased plant productivity and J_{CO2} under
348 increased precipitation inputs (Raich, Potter & Bhagawati 2002; Harper *et al.* 2005; Fay *et al.*
349 2008; Hoover, Knapp & Smith 2016; Deng *et al.* 2017), and proportionally greater responses to
350 precipitation treatments in ANPP than in J_{CO2} (Raich, Potter & Bhagawati 2002; Hoover, Knapp
351 & Smith 2016). Our findings advance prior work by assessing how precipitation-J_{CO2}
352 relationships link to variation in plant productivity. For the genotypes in this study, ANPP and
353 related components including plant height, tiller number, and tiller mass are highly heritable, and
354 responsiveness to precipitation was related primarily to variation in tiller mass (Aspinwall *et al.*
355 2013; Aspinwall *et al.* 2017). Larger tillers resulting from more precipitation are likely supported
356 by larger root systems, yielding improved access to water and greater carbon uptake supporting a
357 greater mass of respiring root tissue..
358 The switchgrass genotypes in our experiment expressed variation in traits representing a
359 continuum across the acquisitive to conservative PES spectrum. The finding of relationships
360 between aboveground responses (LDMC, N_{MASS}, ANPP) and J_{CO2} demonstrate a functional
361 linkage of aboveground with belowground processes that integrates plant carbon acquisition and
362 allocation strategies (Wardle *et al.* 2004; Reich *et al.* 2008; Reich 2014). Plants with traits that
363 promote greater C assimilation, like high N_{MASS}, should be able to transport more C to roots,
364 increasing the contribution to J_{CO2} (Craine, Wedin & Chapin 1999; Wardle *et al.* 2004; Bardgett

365 *et al.* 2013; Deng *et al.* 2017). Similarly, plants with high N_{MASS} often also possess high nutrient
366 roots (Tjoelker *et al.* 2005) that typically respire and decompose rapidly (Tjoelker *et al.* 2005;
367 Metcalfe, Fisher & Wardle 2011; Bardgett, Mommer & De Vries 2014), affecting both the
368 autotrophic and heterotrophic contribution to J_{CO2}.

369 There is increasing recognition of the role of inter-specific variation in plant traits in
370 driving soil respiration rates in natural communities (e.g., Metcalfe, Fisher & Wardle 2011) but
371 the role of intra-specific variation, here provided by our geographic sample of *P. virgatum*, has
372 received little attention. Plant trait variation can influence soil respiration through two main
373 avenues—by driving variation in root respiration and by determining the rate at which soil
374 heterotrophs metabolize plant-derived carbon (De Deyn, Cornelissen & Bardgett 2008). For
375 example, organic matter derived from *P. virgatum* genotypes possessing more conservative leaf
376 economics strategies should decompose more slowly than that from genotypes possessing more
377 acquisitive strategies (Bardgett 2017). Similarly, genotypes with lower root C:N or greater
378 plasticity in root production may have increasing contributions of autotropic respiration to the
379 total J_{CO2} flux with increasing precipitation compared to genotypes with higher C:N or lower
380 plasticity. These are promising avenues for future research on C cycling and soil C sequestration
381 in perennial grasses and candidate bioenergy crops, like *P. virgatum*.

382 Although PES traits provide a critical functional role in supplying C to the processes
383 driving J_{CO2}, we found little evidence that PES traits mediated the relationship between
384 precipitation and J_{CO2}. The PES traits we considered varied little in response to precipitation
385 compared to ANPP, reinforcing previous work (Knapp & Smith 2001; Fay *et al.* 2003; Siefert *et*
386 *al.* 2015; Aspinwall *et al.* 2017; Griffin-Nolan *et al.* 2018; Lü *et al.* 2018). Our findings also
387 extend previous studies within this system: Aspinwall *et al.* (2013) found that differences among

these genotypes in functional trait combinations were correlated with the genotype's ANPP, but suggested that traits reflected adaptation to climate and soils, and were not drivers of productivity *per se*. This study suggests that precipitation variability further decoupled ANPP and PES traits in these genotypes, breaking a potential pathway by which traits may have mediated the effect of precipitation on J_{CO2}. This interpretation is consistent with current theory, which suggests that traits impact the efficiency with which carbon is acquired and allocated to aboveground and belowground processes (e.g., Cornwell *et al.* 2008; De Deyn, Cornelissen & Bardgett 2008; Bardgett, Mommer & De Vries 2014), but in this experiment, the amount of carbon flowing through the plant-soil system yielding J_{CO2} may more strongly reflect resource availability and demand.

We found different patterns relating precipitation, ANPP, and PES traits to J_{CO2} at Austin and Temple. In Austin precipitation explained substantially more variation in ANPP (R^2=0.43 and R^2=0.25, respectively), consistent with previous findings from this experiment (Lovell *et al.* 2016; Aspinwall *et al.* 2017). Consequently, the effect of precipitation on J_{CO2} was more strongly mediated by ANPP in Austin than Temple. Despite this, precipitation explained similar amounts of variation in J_{CO2} at both sites. The tighter coupling of ANPP and J_{CO2} with precipitation at Austin is consistent with the more coarse-textured and shallower soils and lower overall soil moisture compared to Temple and align with previous findings of tighter coupling of ANPP to moisture availability on coarse-textured soils (Fay *et al.* 2012). The sites were also similar in precipitation treatments, temperature, and evaporative demand, pointing to edaphic factors as a potential cause of site differences in the drivers of J_{CO2}. However, because other potentially important factors, like nutrient availability, have not been accounted for, we cannot mechanistically explain differences between these sites.

PES traits also played a larger, but still minor, role in mediating precipitation effects on J_{CO2} at Austin, suggesting that traits related to C gain matter more in regulating ecosystem function when water is more limiting. In contrast, at the Temple site, where water was less limiting to plant growth, the link between precipitation and J_{CO2} became decoupled from plant traits (Curtin, Beare & Hernandez-Ramirez 2012). Together, these results support previous findings (Bouma & Bryla 2000; Risch & Frank 2006) showing that edaphic differences can create variation in the contribution of plant functional traits and productivity to precipitation effects on J_{CO2}.

The scope of this study did not include partitioning the heterotrophic and autotrophic components of J_{CO2}. Existing studies from both forest and grassland ecosystems generally report increases in both components with increased precipitation (Li *et al.* 2018; Zhang *et al.* 2019; Song *et al.* 2020, but see Liu et al. 2016). Changes in the balance between the two respiration components under increased precipitation will likely depend on the way plants allocate resources. With increased precipitation, plant allocation to root biomass may decrease relative to allocation aboveground, decreasing the autotrophic component while the heterotrophic component increases because microbial metabolism increases with soil moisture in non-saturated soils (Zhang *et al.* 2019). We predict that the shift toward heterotrophic respiration would be greater at Temple, where water was less limiting to productivity. A relatively greater contribution of autotrophic respiration to the increase in J_{CO2} at Austin would also be predicted by the increase in BNPP with precipitation.

We found BNPP and belowground traits to be poor predictors of J_{CO2}. Root C:N increased with precipitation, indicating lower decomposability, yet this trait did not influence J_{CO2}. Instead, autotrophic respiration and heterotrophic respiration of labile C may have

overwhelmed heterotrophic respiration of root litter. BNPP may have been a poor measure of plant size because it only accounted for new growth, not the size of standing root mass. Additionally, plant belowground traits are often strongly linked to mycorrhizal networks, which we did not measure (Wallenstein & Hall 2012; Bardgett, Mommer & De Vries 2014). These and other limitations of this study provide opportunities for future work.

Conclusions

This study shows that increasing J_{CO2} in response to increasing precipitation is primarily mediated by ANPP, but J_{CO2} can also increase independently of precipitation when plants possess traits, in this case increased N_{MASS}, conferring a more acquisitive resource allocation strategy. Therefore, the differing ways PES traits, plant size, and precipitation combined to predict J_{CO2} has implications for understanding ecosystem function under changing precipitation regimes. Combining estimates of ANPP with PES traits may also improve estimates of the contributions of J_{CO2} to C cycling in more resource-limited environments.

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Data accessibility: Upon acceptance, data will be archived in Dryad.

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Table 1 Effects of precipitation on soil respiration (J_{CO2}) mediated by plant economics traits (N_{MASS}, LDMC, root C:N), plant size (ANPP, BNPP), and an *abiotic* factor (soil temperature) from structural equation models. Indirect effects show the effect of precipitation from paths that are solely mediated by an endogenous variable within that category. Effects that were mediated by both ANPP and soil temperature were considered abiotic. The total effect of precipitation on J_{CO2} is the sum of all significant paths from precipitation to J_{CO2} that are mediated by one or more endogenous variables plus the direct effect of precipitation. NS indicates no significant paths in a given category.

	Total precipitation effect	Precipitation effect on J_{CO2} mediated by:		
Site		Plant Economics	Plant Size	Abiotic
Austin	0.57	-0.06	0.28	0.36
Temple	0.62	NS	0.09	NS

Table 2 Relative importance of aboveground (ANPP, N_{MASS}, LDMC) and belowground (root C:N, BNPP) plant traits in predicting soil respiration (J_{CO2}). To calculate relative variable importance (RVI), all possible models containing additive effects of these five traits were ranked by AICc. For each predictor, RVI is the sum of Akaike weights of all models including that predictor.

	Relative importance predicting J_{CO2}	
	Austin	Temple
ANPP	1	0.77
LDMC	0.94	0.72
N_{MASS}	0.85	0.71
Root C:N	0.77	0.46
BNPP	0.22	0.3

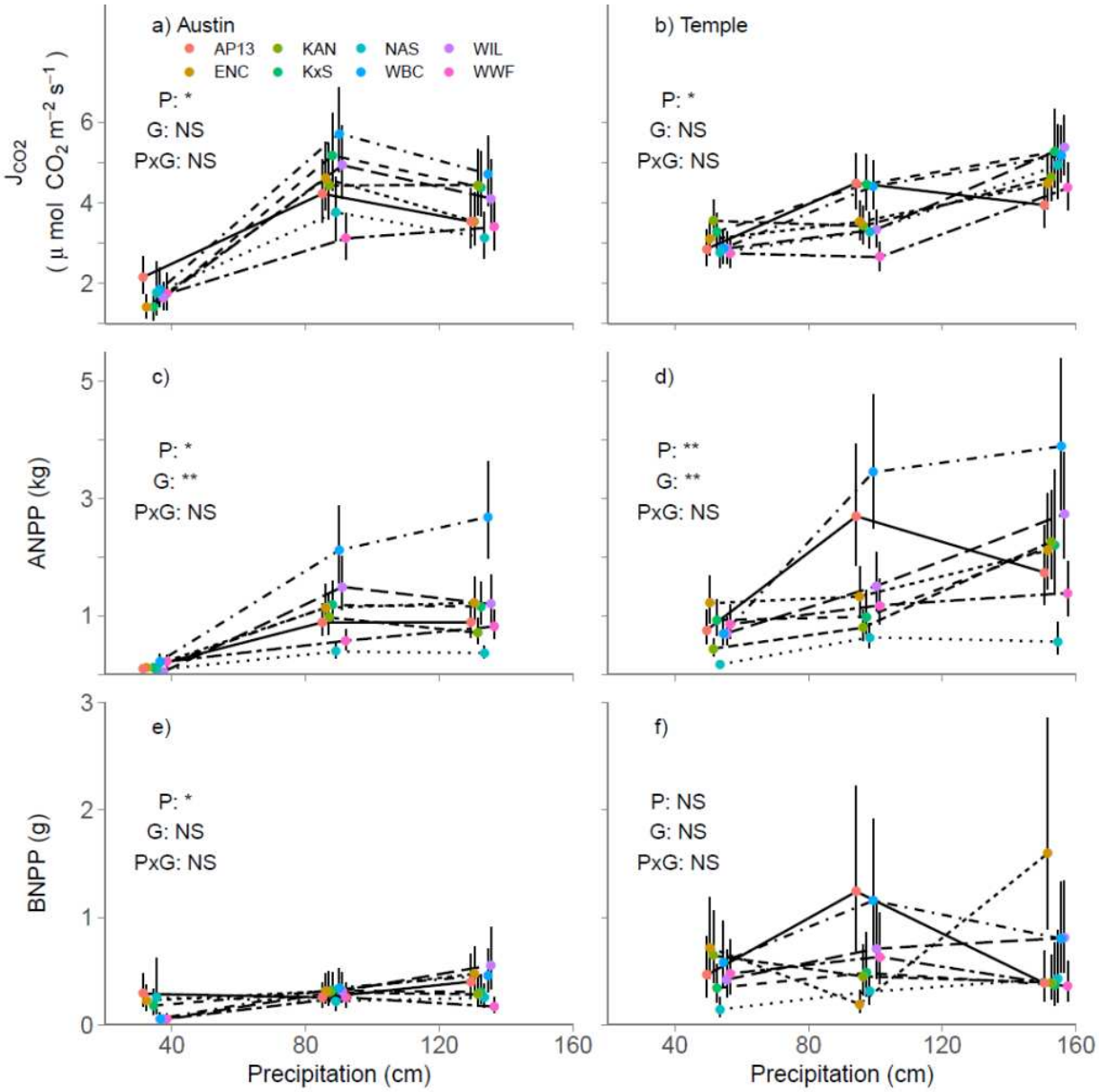

Fig. 1 Effects of plant genotypes and precipitation treatment on **a**, **b** soil respiration (J_{CO2}); **c**, **d** ANPP; **e**, **f** BNPP. Results from Austin are in the left column and those from Temple are in the right column. Points denote mean ± standard error. For each treatment (precipitation: P, genotype: G, and precipitation × genotype: PxG), P values <0.001 are denoted **, 0.05 > P value > 0.001 are denoted *, and P value > 0.05 are denoted NS.

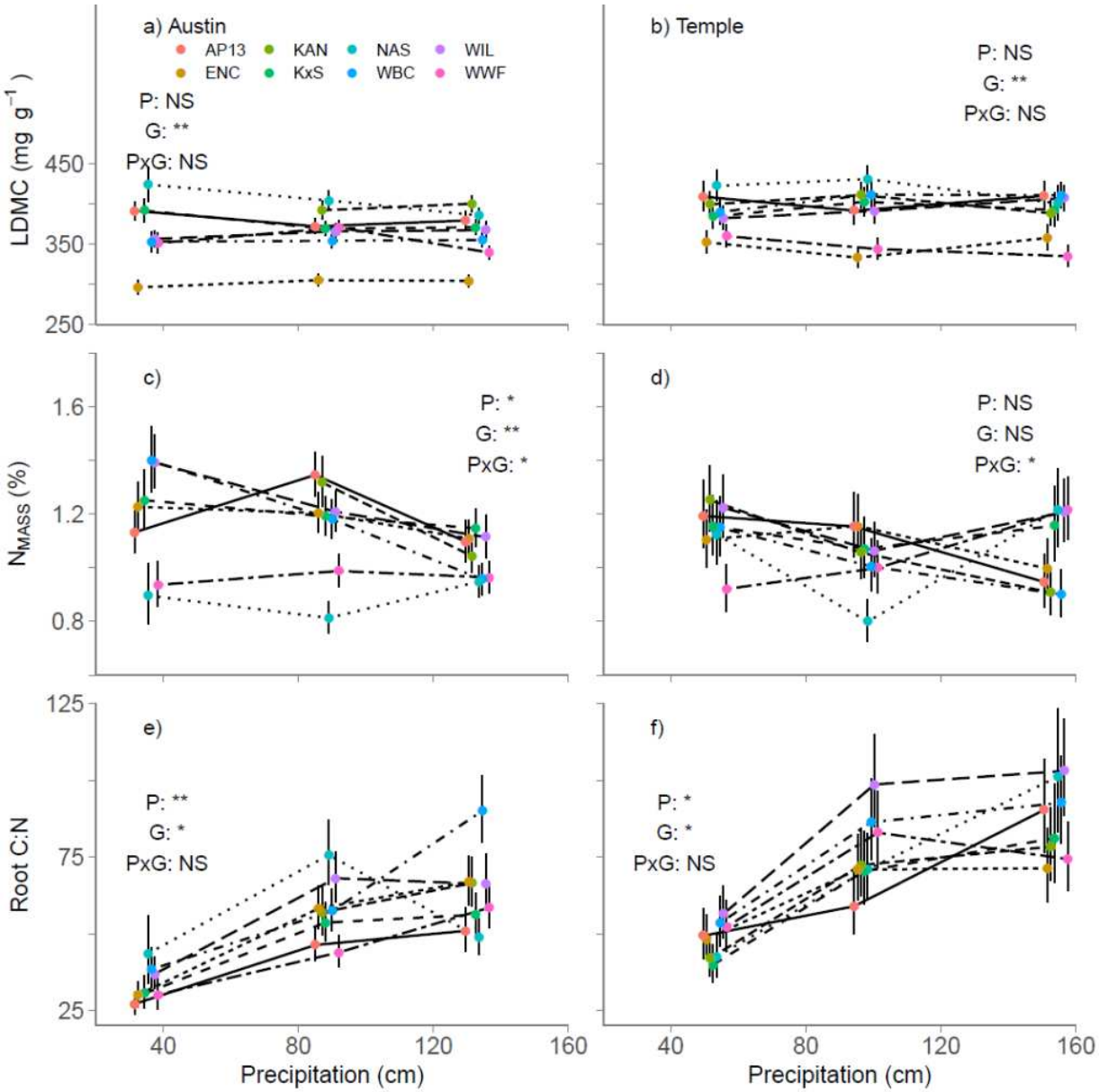

Fig. 2 Effects of plant genotypes and precipitation treatment on **a**, **b** leaf dry matter content (LDMC); **c**, **d** foliar N_{MASS}; **e**, **f** root C:N. Results from Austin are in the left column and those from Temple are in the right column. Points denote mean ± standard error. For each treatment (precipitation: P, genotype: G, and precipitation × genotype: PxG), P value < 0.001 are denoted **, 0.001 ≤ P value < 0.05 are *, and P value > 0.05 are denoted NS.

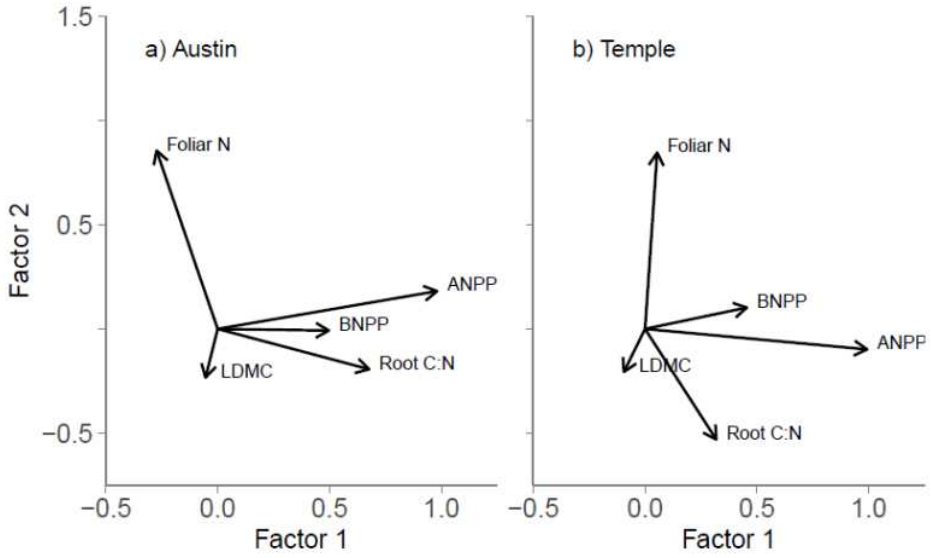

Fig. 3 Factor score biplots showing the relationship between five plant traits—foliar N_{MASS}, leaf dry matter content (LDMC), root C:N, ANPP, and BNPP—from exploratory factor analysis at **a** Austin and **b** Temple.

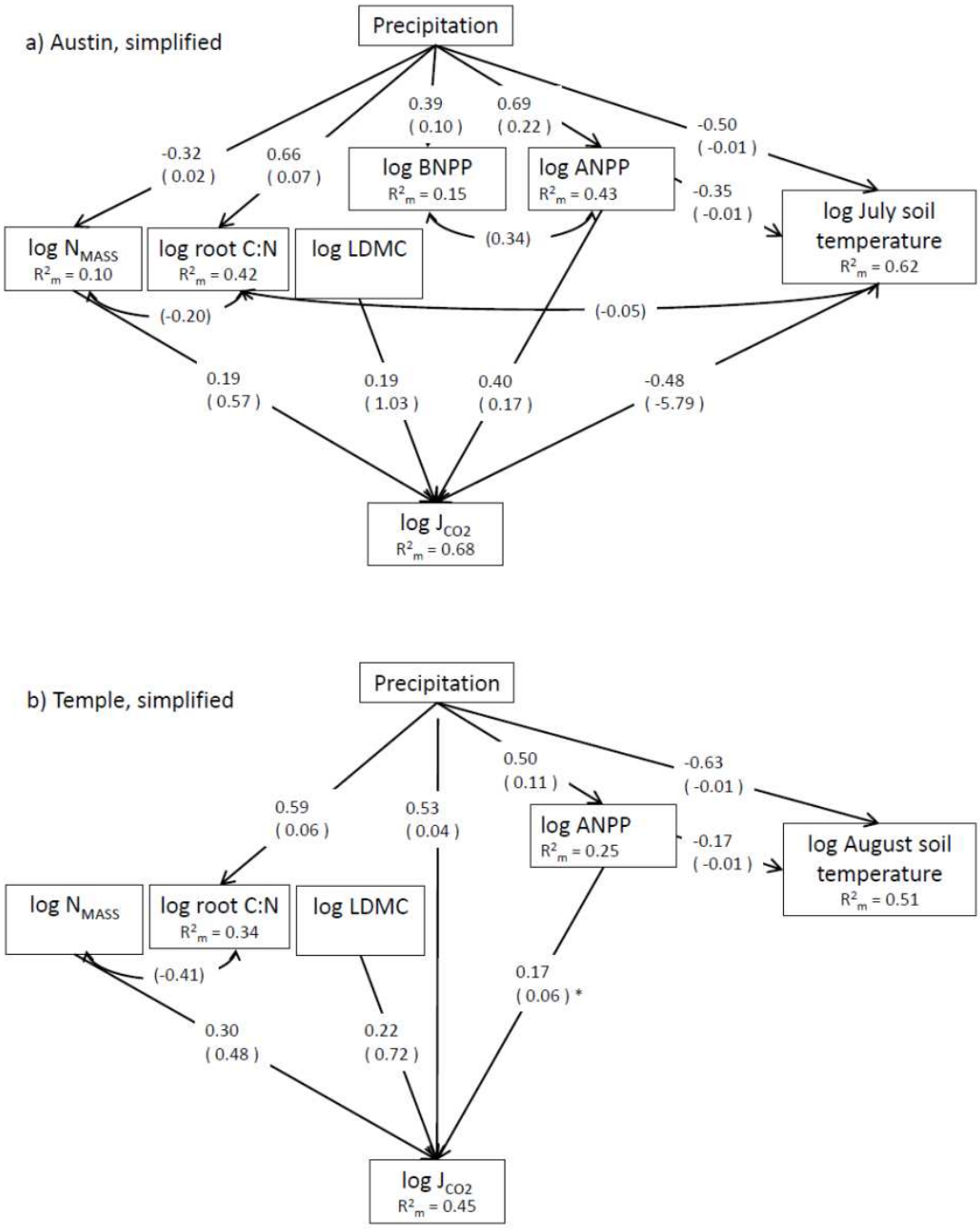

Fig. 4 Piecewise structural equation model with non-significant paths removed, **a** Austin, **b** Temple. Each path shows standardized coefficients with unstandardized coefficients in parentheses. Double-headed arrows denote correlated errors. For each endogenous variable, R^2 represents the variance explained by fixed effects in the model (marginal R^2). A marginally significant effect ($0.05 \leq P < 0.1$) is represented by *.